Effect of pelleting temperature, probiotic and wheat grain on intestinal pH, cecal microbial population and intestinal morphometry in broiler chickens

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Abstract

The main objective of the present study was to examine the effects of dietary probiotic and wheat inclusion and pelleting die temperature (70 or 85 °C) on intestinal pH, cecal microbial population, and intestinal morphology. A 2×2×2 factorial arrangement in the completely randomized design was applied that included wheat inclusion, probiotic supplementation and conditioning temperature as the main factors. Wheat addition significantly increased pH of jejunum content. Probiotic supplementation and die temperature had no main and interaction effects on pH of digesta. There was no significant difference for main effects of different factors on the population of lactobacillus and Bacillus counts. The interaction between probiotic supplementation and die temperature had a significant effect on the population of lactobacillus and Bacillus counts. Wheat inclusion increased, probiotic supplementation decreased and higher die temperature increased the population of *E. coli* in the cecal content. Main effects and interactions were not different on jejunal villus height, crypt depth and villus height: crypt depth ratio. Probiotic supplementation increased ($P<0.05$) and higher die temperature decreased the goblet cell count. It was suggested that inclusion of low non starch polysaccharide wheat grain as pellet binder and energy source can perform alone or in combination with probiotics at die temperature of 70 °C.

Keywords: Probiotic; Wheat; Die temperature; Villi; Chicken

Introduction

In the poultry feed industry, the cost effective and the most widely used form of heat processing method is pelleting (Abdollahi et al., 2010a; Amerah et al., 2011). Feeding pelleted diets has advantages on the performance of broiler chickens (Calet, 1965; Douglaset al., 1990; Jensen, 2000), higher feed density, improved digestibility, increased nutrient intake, reduced feed wastage and decreased energy spent for eating (Abdollahi et al., 2010a; Calet, 1965; Jensen, 2000).

Conditioning is a major step of pelleting in which steam added to the mash to be pelleted (Skoch et al., 1981). The wide ranges of conditioning temperature are used in the feed manufactures (McCracken, 2002). If the conditioning step was not done in the appropriate temperature especially in wheat based diets, the negative results of feeding pelleted diets to broilers, such as poor performance, appear. Moreover, pelleting has another disadvantage like loss of amino acids through the Millard reaction, starch retrogradation and resistance, increase in viscosity, loss of heat labile vitamins and live organisms additives, such as probiotics (Abdollahi et al., 2010b; Jensen, 2000; McCracken, 2002). Some probiotics based bacillus strains are heat stable and have advantages in broiler performance and health (Majidi-Mosleh et al., 2017). A scarcity information exist concerning the ability of probiotics to survive in steam-pelleting conditions. Also, interactions of probiotic supplementation with different pelleting temperatures with or without wheat grain as pellet binder on intestinal pH, cecal microbial population and intestinal morphology are also unknown. Therefore, the main objective of the present study was to examine the effects of the supplementation with probiotic based on *Bacillus subtilis*, pelleting temperature with or without wheat grain on gastrointestinal pH, cecal microbial population and jejunal morphology.

Materials and methods

Chickens and experimental design

This experiment was conducted in summer 2017 at Animal Research Center (Karaj, Iran) with an altitude of 1160 m above sea level, the latitude of 35°47′ N and longitude of 51°08′ E. The chemical material and culture media were prepared from Merck Company (Dusseldorf, Germany) and laboratory activities were done in Razi Lab Complex (Tehran, Iran).

Eight hundred one-day-old broiler chicks (Cobb 500 strain) were purchased from a commercially local hatchery. Chicks were raised in the floor pens under environmentally controlled conditions and lighting program based on Cobb 500 broiler guides. Diets were formulated based on the requirements of Cobb 500 broiler chickens. The same starter ration was given to chicks until day 10 of age. At day 11 of age, chicks were individually weighed, and assigned to pens (12 birds per pen). A 2×2×2 factorial arrangement in the completely randomized design was applied that included wheat inclusion (0 or 50%), probiotic supplementation (0 or 200 mg/kg) and conditioning temperature (70 or 85 °C) as the main factors.

Probiotic sample was prepared from TakGen Company (Tehran, Iran) with the trade name of Bactogen® containing *Bacillus subtilis* (JQ61816).

Chicks were assigned to 8 treatments and four replicates and fed dietary treatments until day 42 of age. Feedstuffs were ground, blended in mixer and conditioned at 70 or 85 °C for 45 s and pelleted with a ring die pellet set.

Sample collection and measurement

At day 25 of age, 8 chicks (two chicks per replicate) were randomly chosen from each treatment, and then euthanized by cervical dislocation. Immediately, the digestive tract was eviscerated and the pH of different parts of gastrointestinal tract was recorded using the pH meter that directly inserted into the digesta. Also, caecal contents of 8 chicks per treatment (two chicks per each replicate) were collected, pooled and a sub-sample was taken to assay the populations of lactobacillus, *Bacillus subtilis* and *E. coli*. The populations of bacteria were estimated as the log 10 of colony forming units (cfu) per gram of content. Then the jejunum of these chicks were excised and segments washed with cold phosphate buffer saline and then fixed in 10% buffered formalin solution for histological processing.

Culture

One gram of each sample was ten-fold serially diluted in 0.9% sterile bacteriological peptone diluents. Lactobacillus and *Bacillus subtilis* were cultured on MRS agar in aerobic condition. *E. coli* was counted by EMB agar (Eosin methylene blue). All commercial media (Merck, Dusseldorf, Germany) were rehydrated in distilled water. The agar media were sterilized by autoclaving at 121 °C for 15 min. All the cultured samples were incubated in 37 °C after 48 h, the plates containing 25 to 250 colonies were

enumerated and recorded as cfu/g of sample. The number of replicate samples was three, and the experimental program was repeated twice.

Intestinal morphological analysis

Formalin fixed samples were dehydrated by passing through graded alcohol solutions and the cleared by passing thorough xylene, and embedded in paraffin. Sections (6-µm) were prepared from each segment using a microtome and sections were placed on glass slide. Mayer´s hematoxylin and eosin routine procedure was applied for staining of the slides. Villus height and length, and crypt depth were measured according to the method of Iji et al. (2001) using a light microscope. The method of Horn al. (2009) was used for measurement of the goblet cell counts in the scale of 300 µm of epithelium length.

Statistical Analysis

Firstly, the Kolmogorov–Smirnov test was done to check the normality of data distribution. The data was analyzed with ANOVA of SAS using GLM procedure for Windows version 14.1 as a 2×2×2 factorial arrangement of treatments in completely randomized design that included conditioning temperature, prebiotic supplementation and wheat inclusion as the main factors and their respective interactions. Means were separated by Tukey test at $P \leq 0.05$.

Results and Discussion

Many studies have suggested that the benefit of a probiotic as growth stimulation of chicks would be the result of an enhancement in the digestive system and intestinal environment and health (Tellez et al., 2006; Mountzouris et al., 2010). In addition to the intestinal environment (pH and bacterial population), and intestinal health (morphometry), the determination of goblet cells count under influence of wheat inclusion in the diet, Probiotic supplementation and die temperature during pelleting were the main objectives of this study.

As presented in Table 1, significant difference existed for main effects of wheat addition to diet on pH of jejunum contents, but there was no significant difference for main effects and also interactions of wheat on pH of other gastrointestinal parts.

Wheat addition significantly increased pH of jejunum content. Probiotic supplementation and die temperature had no main and interaction effects on pH of digesta. The wheat cultivar used in this study as pellet binder had the minimum contents of non-starch polysaccharide, thus its effect on the passage rate of digesta and consequently pH of digesta was insignificant.

Table 1. The main and interaction effects of wheat, probiotic and die temperature on pH of digesta

	Proventriculus	Gizzard	Duodenum	Jejunum	Ileum
Main effect					
Wheat					
0	3.14	2.42	5.28	4.96 b	5.58
500	3.01	2.45	5.18	5.21 a	5.46
Probiotic					
0	3.10	2.40	5.30	5.13	5.59
200	3.06	2.46	5.15	5.06	5.45
Temp					
70	2.99	2.40	5.27	5.07	5.52
85	3.16	2.43	5.18	5.11	5.51
P value					
Main effects					
Wheat	0.584	0.813	0.462	0.049	0.393
Probiotic	0.857	0.654	0.256	0.521	0.282
Temp	0.485	0.963	0.481	0.785	0.898
Interactions					
W×P	0.382	0.015	0.966	0.853	0.974
W×T	0.136	0.502	0.410	0.586	0.665
P×T	0.124	0.906	0.779	0.627	0.517
W×P×T	0.762	0.129	0.440	0.118	0.332

[a, b] Means within a column with different superscripts are significantly different ($P<0.05$).

In the jejunum of chicks, pancreatic and bile secretions can greatly influence on digesta pH (Dahlke et al., 2003). Wheat addition to diet may positively influence on the pancreatic and bile secretions and increase the digesta pH. As presented in Table 2, the factors used in this study, especially

wheat addition to diet, had no effect on the population of Lactobacillus. The population of Lactobacillus could influence on the digesta pH.

The effects of treatments on bacterial population are shown in Table 2. There was no significant difference for main effects on the population of lactobacillus and Bacillus counts. The interaction between probiotic supplementation and die temperature had a significant effect on bacterial population of lactobacillus and Bacillus counts. Significant differences exist for the main effects on population of *E. coli*. Wheat inclusion increased, probiotic supplementation decreased and higher die temperature increased the population of *E. coli* in the cecal content. The interaction between different factors was not significant for population of *E. coli*, except for wheat × die temperature.

Table 2. Bacterial population (log_{10}/g) of cecal contents of chicks in different treatments

	Lactobacillus	*E. coli*	Bacillus
Main effect			
Wheat			
0	13.55	8.25 b	6.01
500	13.23	8.79 a	5.83
Probiotic			
0	13.19	9.10 a	5.95
200	13.56	7.99 b	5.89
Temp			
70	13.47	8.00 b	5.95
85	13.30	9.02 a	5.89
P value			
Main effects			
Wheat	0.274	0.024	0.106
Probiotic	0.220	0.001	0.525
Temp	0.570	0.001	0.547
Interactions			
W×P	0.985	0.714	0.826
W×T	0.848	0.040	0.900
P×T	0.006	0.546	0.442
W×P×T	0.416	0.358	0.416

[a, b] Means within a column without different superscripts are not significantly different (P>0.05).

Table 3. Intestinal morphometry (mm) of chicks received different treatments

	Height	Width	Depth	ratio	Goblet cells
Main effect					
Wheat					
0	1.17	0.193	0.133	14.32	9.46
500	1.16	0.130	0.085	13.87	9.87
Probiotic					
0	1.16	0.191	0.136	14.96	9.20 b
200	1.16	0.131	0.078	13.26	10.12 a
Temp					
70	1.17	0.195	0.138	13.64	10.20 a
85	1.15	0.128	0.080	14.51	9.18 b
P value					
Main effects					
Wheat	0.371	0.337	0.378	0.164	0.293
Probiotic	0.732	0.356	0.312	0.647	0.0.21
Temp	0.108	0.309	0.299	0.089	0.018
Interactions					
W×P	0.096	0.301	0.323	0.085	0.800
W×T	0.245	0.346	0.317	0.411	0.456
P×T	0.051	0.327	0.360	0.190	0.438
W×P×T	0.277	0.309	0.313	0.176	0.590

[a, b] Means within a row with different superscripts are significantly different (P<0.05).

In this study, lactobacillus and *Bacillus subtilis* populations were numerically lower in groups received wheat. This event may be resulted in significant increase in the *E. coli* population. Lactobacilli populations can prevent the colonization of *E. coli* (Cribby et al., 2008) and as seen in Table 2, addition of wheat to diet had negative effect on the intestinal health of chicks. In the literature, there was no study about the effect of wheat addition on intestinal bacterial population in broiler chickens.

Main effect of probiotic supplementation on *E. coli* was significant, but its effect on beneficial bacteria was not significant. Interactions were not significant. Probiotic supplementation decreased *E. coli* population. It has been speculated that probiotics have a positive effect on population of intestinal beneficial microflora and negative effect on pathogens (Kabir, 2009; Ohh, 2011). Indigenous anaerobic bacteria as probiotics can limit the population of pathogenic bacteria (mostly aerobic) in the digestive tract (Vollaard et al., 1994) via competitive exclusion.

Main effects and interactions were not different on jejunal villus height, crypt depth and villus height: crypt depth ratio (Table 3). Probiotic supplementation increased ($P<0.05$) and higher die temperature decreased the goblet cell count.

The intestinal digestive function is related to intestinal morphology and it closely related to the intestinal environment and microflora (Liu et al., 2008). Many studies (Awad et al., 2009) showed that probiotic supplementation resulted in an increase in the intestinal villus height. In this study, probiotic supplementation had no effect on the intestinal morphometry. Probiotic supplementation in this study increased the count of goblet cells. This finding is consistent with Rahimi et al. (2010) and Smirnov et al. (2005) who found that supplementation of lactic acid based probiotics can increase the goblet cell density and size.

The findings of the present study suggest that inclusion of low non starch polysaccharide wheat grain as pellet binder and energy source can perform alone or in combination with probiotics at die temperature of 70 °C. The population of *Escherichia coli* is an issue in diet based on wheat grain and application of higher die temperature from 70 °C can increase the population of *Escherichia coli* and finally induce the intestinal health problems.

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